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HORMONAL AND SOCIAL FACTORS INFLUENCING ALTERNATIVE MATING
TACTIC DECISIONS IN MALE GREEN TREEFROGS, *HYLA CINEREA*

by
Joseph Brooks Pratt

A thesis submitted to the faculty of The University of Mississippi in partial fulfillment of the
requirements of the Sally McDonnell Barksdale Honors College

Oxford
May 2016

Approved by

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ABSTRACT

In many anuran species (frogs and toads), males alternate between calling and a non-calling “satellite” mating tactic. Satellite males characteristically associate with calling males and attempt to intercept females that are attracted to the vocalizations of the calling “host” male. The prevailing hypothesis is that the decision to adopt a calling or satellite mating tactic is based on relative attractiveness – males are predicted to adopt a satellite mating tactic when the vocalizations of nearby males are more attractive than their own. In support of this hypothesis, previous studies have shown that satellite males can be induced to call by removing the associated calling host male. However, there is considerable variation in the responses of males in host removal experiments, suggesting that the physiological status of males may play a central role in tactic decisions. For my honors research, I investigated the role of circulating hormone levels in mediating mating tactic decisions in the green tree frog, *Hyla cinerea*. I performed host removal experiments wherein calling males were removed in natural satellite-caller associations. I then observed whether satellite males adopted a calling tactic or remained as non-calling males and subsequently obtained blood samples for hormone analysis. Based on recent models describing the hormonal basis for calling behavior in anurans, I predicted that satellite males would have lower levels of circulating androgens, higher levels of stress hormones, be in poorer condition, and produce calls that are less attractive than calling males. Moreover, satellite males that did not call subsequent to removal of the calling host male were predicted to have lower circulating androgen levels and higher levels of CORT than satellite males that called subsequent to removal of the calling host male. Consistent with these predictions, satellite male *H. cinerea* had lower levels of dihydrotestosterone (DHT), higher levels of corticosterone (CORT), were in poorer condition and invested less energy in their calls than calling host males. Satellite males

that called subsequent to host removal also had significantly higher DHT levels; however, circulating CORT levels did not differ for the two groups of males. My results suggest that circulating androgen levels play a central role in mediating mating tactic decisions in *H. cinerea*.

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LIST OF ABBREVIATIONS

EHV	Energetics-Hormone Vocalization
CORT	Corticosterone
T	Testosterone
DHT	Dihydrotestosterone

INTRODUCTION

Competition among males for females can lead to the evolution of diverse sexual behaviors, collectively referred to as alternative mating behaviors (Gross, 1996). Behavior serves as a mediator between an organism's internal and external environment, and reproductive success is determined, in part, by the proper synchronization of an organism's activities and its internal and external environments (Crews and Moore, 1986). The mechanisms that are linked to the physiological, social, and environmental cues that trigger the plethora of different sexual behaviors result from environmental challenges that organisms face (Crews and Moore, 1986) and alternative mating behaviors appear to evolve in response to such challenges (Alonzo and Sinervo, 2001).

Alternative male mating behaviors may arise as a consequence of genetic polymorphisms that result in two or more fixed alternative phenotypes that persist throughout the lifetime of the organism, often termed "strategies" (Gross, 1996). Alternatively, variation in mating behavior can arise among genetically monomorphic individuals that conditionally alternate between two or more plastic alternative phenotypes, often termed "tactics" (Brockman, 2001; Gross, 1996). However the extent to which differences in male mating behavior relate to genotypic differences are often unclear (Taborsky et al. 2008). For example, discontinuous variation is observed among males of several species of horned beetles with respect to the presence of horns; large males possess large horns and small males lack horns or possess rudimentary horns. Large males use horns in combat for access to females whereas small males utilize alternative, less aggressive mating strategies (Emlen, 1997). In contrast to this fixed behavioral phenotype, male horseshoe crabs arrive at nesting beaches either attached (mounted) to females or as solitary unattached males; unattached males gather around paired "couples" and attempt to fertilize eggs via sperm

competition and may alternate between these two tactics (Brockmann and Penn, 1992). Whether such differences in behavior arise as a consequence of discrete genetic polymorphisms or gene-environment interactions among genetically monomorphic individuals remains unknown.

Phenotypic plasticity is defined as an environmentally-based change in an organism's phenotype (Via et al., 1995) and can confer distinct fitness advantages (DeWitt et al., 1998). Morphological variation is often related to variation in sexual behavior. For example, differences in tail length are related to alternative mating behaviors of male guppies (*Poecilia reticulata*). Males of this species with longer tails are less frequently observed participating in cooperative copulation and are more often observed trying to sneak attempts at forced copulation (Karino and Kamada, 2009). However, phenotypic plasticity is not limited to variation observed in physical characteristics - it also extends to environmentally based changes in an organism's physiology (Kelly et al., 2012).

Sexual behaviors are often regulated by hormones that can play a central role in mediating the expression of plastic alternative mating tactics (Crews and Moore, 1986; Moore, 1991; Thompson and Moore, 1992). For example, the Relative Plasticity Hypothesis proposes that hormones influence the expression of reproductive phenotypes via organizational and/or activational effects (Phoenix et al. 1959; Moore et al. 1998). Organizational effects of hormones typically occur early in development during a critical period and mediate irreversible changes in the phenotype (Moore, 1991). In contrast, activational effects of hormones typically occur after the organism is sexually mature and mediate changes in the phenotype that are temporary and reversible and coincide with variation in circulating hormone levels (Moore, 1991). Based on these ideas, the Relative Plasticity Hypothesis distinguishes between "fixed" and "plastic" morphs that differ in whether the phenotype is subject to organizational or activational effects of

hormones; organizational effects of hormones are predicted to be critical in determining the differences in “fixed” morphs while activational effects of hormones are predicted to be critical in establishing the differences between males with “plastic” alternative male phenotypes (Thompson and Moore, 1992).

Support for these predictions comes from extensive work on tree lizards, *Urosaurus ornatus*. In this species, there are two fixed phenotypes: orange and orange-blue males that differ in the color of their dewlap (Thompson and Moore, 1992). Progesterone appears to be of primary importance in mediating organizational effects that are essential for the differentiation of these two fixed morphs (Jennings et al. 2003). For example, males given a single injection of progesterone after hatching (Moore et al., 1998) develop into the orange-blue morph whereas exposure to low levels of progesterone on the day of hatching will cause the male to develop into the orange morph (Roughgarden, 2013). In contrast, corticosterone (CORT) and testosterone (T) play an activational role in the mediation of alternative mating tactics in the orange morph (Thompson and Moore, 1992). In this morph, reciprocal interactions between the two steroids mediate a nomadic mating tactic where individuals have no fixed home range and move among the territories of several males (Knapp et al., 2003). On the other hand, the orange-blue morph does not show a reciprocal interaction between circulating CORT and T levels and maintains a site-faithful mating tactic wherein territories are aggressively defended (Knapp et al., 2003).

The Relative Plasticity Hypothesis has been incredibly influential in directing research aimed at understanding the underlying physiological mechanisms regulating alternative mating tactics and numerous studies have found differences in circulating hormone levels among males that adopt different mating tactics (Knapp et al., 2003; Leary and Harris, 2013; Brantley et al., 1993). However, it is often unclear whether the differences in circulating hormone levels in

males practicing different mating tactics are a cause or consequence of differences in behavior (Oliveira et al., 2008). For example, circulating hormone levels may not only influence behavior, but can also be influenced by an organism's social environment (Oliveira et al., 2008). Investigation of this problem could provide insight into the role of hormones versus the social environment in mediating alternative mating behaviors.

Alternative mating behaviors in anurans (frogs and toads) are a model in which to address this problem. Males of many anuran species conditionally alternate between a calling and non-calling "satellite" mating tactic (Waltz, 1982). Non-calling satellite males characteristically remain in close proximity to calling males and attempt to intercept females attracted to the calls of the calling "host" male (Halliday and Tejedo, 1995). Upon removal of the calling host male from a satellite-caller association, satellite males often switch their mating tactic and begin vocalizing (Perrill et al., 1982). The prevailing hypothesis to explain the adoption of calling behavior in satellite males subsequent to removal of the calling host is that mating tactic decisions are based on the relative attractiveness of other males – males are predicted to adopt a satellite mating tactic when the vocal signals of nearby males are more attractive than their own. In support of this hypothesis, satellite male toads (*Bufo cognatus*, *B. woodhousii*) and green treefrogs (*H. cinerea*) tend to associate with calling host males producing more attractive calls (Leary et al, 2006; Humfeld, 2008). However, it is not clear whether the relative attractiveness of other males explains the variation in calling behavior observed in host removal experiments, as some satellite males do not call subsequent to the removal of the calling host and maintain the satellite tactic in the absence of another male's vocal signals. In *Hyla cinerea*, for example, removal of the calling host male resulted in transitions to calling behavior in satellite males only in approximately 50% of 19 host removal experiments (Perrill et al., 1982).

Circulating hormone levels may explain the variation in mating behavior observed among satellite males subsequent to removal of calling host males. For example, satellite male green treefrogs (*Hyla cinerea*) have lower plasma dihydrotestosterone (DHT) and testosterone (T) levels and higher plasma corticosterone (CORT) levels than calling males (Leary and Harris, 2013). These differences in circulating hormone levels in caller and satellite males are consistent with predictions of the Energetics-Hormone Vocalization model that was proposed to explain the hormonal basis for transitions between calling and non-calling behavior in anuran amphibians (Emerson, 2001). The model proposes that interactions among competing males result in increases in circulating androgen levels that mediate an increase in vocal effort. Vocalization is energetically expensive (Taigen and Wells, 1985) and the energetic requirements associated with increased vocal effort are expected to stimulate the production of CORT to mobilize energy reserves (Emerson, 2001). Elevated CORT levels, resulting from the depletion of energy reserves, are predicted to negatively affect circulating androgen levels and suppress vocalization (Emerson, 2001). In contrast, differences in circulating hormone levels may also be a consequence of tactic expression. For example, vocal playback studies showed that acoustic signals produced by male *H. cinerea* can stimulate CORT production and lower androgen levels in conspecific male receivers (Leary, 2014).

For my honors thesis I performed a host removal experiment using male green tree frogs (*Hyla cinerea*) to examine the extent to which variation in the behavioral responses of satellite males are related to relative vocal attractiveness and circulating hormone levels. I studied several vocal attributes known to be important in mate selection and male-male competition (Humfeld, 2008; Leary et al. 2014) including carrier frequency, call duration, intercall duration, and vocal effort. Female *H. cinerea* prefer vocalizations with a lower frequency in the low frequency peak

of the advertisement call (Humfeld, 2008) and calls that are longer in duration (Gerhardt, 1988). Hence, I predicted that satellite males would produce calls that are less attractive to females. I also examined circulating levels of T, DHT, and CORT to address the endocrine differences between calling and satellite males and also between satellite males that called subsequent to the removal of the calling host and those that did not. Based on the Energetics-Hormone Vocalization model (Emerson, 2001) I predicted that satellite males would have lower levels of circulating androgens and higher circulating levels of CORT relative to calling host males and that satellite males that called subsequent to host removal would have higher circulating androgen levels and lower CORT levels than satellite males that do not call subsequent to host removal. If hormones are a primary factor in mediating mating tactic decisions in *H. cinerea*, I expected to find differences in hormone profiles for the two groups of satellite males (i.e., those that call and those that do not call subsequent to host removal). However, if tactic decisions are mainly governed by the relative attractiveness of other males, I expected to find no significant endocrine differences in the two groups of satellite males.

METHODS

Data collection

Data was collected from natural choruses of green treefrogs, *H. cinerea*, from May through July at the University of Mississippi Field Station in Abbeville, Mississippi (34.425°N, -89.392°W). Observations and experiments occurred between 20:30 and 24:00 h.

Caller-satellite relationships were located using LED headlamps (which didn't have any noticeable effects on behavior) and observed for a period of at least 10 minutes to ensure stable caller-satellite associations. Calling hosts and satellites were in close proximity to each other (about 1 meter) and satellites did not call during the observation period. Approximately 30 calls of the calling host male were recorded during the observational period using a Marantz PMD-222 recorder equipped with a Sennheiser ME66 condenser microphone. The calling host male was then removed by hand from the vicinity of the satellite male and blood was immediately collected from the calling male with a sterile 27 gauge heparinized hypodermic needle and syringe in less than 5 minutes via cardiac puncture. Blood samples were stored on ice after collection. The satellite male was then observed for 15 min to determine if it called subsequent to removal of the calling host male. For the satellite males that vocalized, approximately 30 advertisement calls were recorded. Blood samples were then obtained via cardiac puncture. For the satellite males that did not vocalize during the 15 minute time frame, the same procedures were carried out, excluding recording of vocalizations. Males were then weighed using a portable OHAUS scale, measured from the snout to ischium (snout-ischial length, SIL) to the nearest millimeter. Males were then marked using Tattoo-A-Pet, a portable tattoo device (Fort Lauderdale, FL, U.S.A.) and released. Upon returning to the lab, blood samples were centrifuged

at 2200 rpm to separate the plasma, which was stored at -20 °C until radioimmunoassay was performed.

Vocal Analysis

Vocal analysis of the calls of both satellite males and calling host males was performed by utilizing Raven Pro 1.4 software (Cornell Lab of Ornithology Bioacoustics Research Program, Ithaca, NY, U.S.A.). Several call parameters important to mate selection and male-male competition (Gerhardt and Huber, 2002; Humfeld, 2008; Leary et al. 2014) were analyzed by studying waveforms, including low frequency, call duration, and intercall duration. Vocal effort was calculated by using the average values from 10 consecutive calls with the following equation:

$$\frac{\text{duration of call}}{\text{Vocal Effort} = \text{duration of call} + \text{intercall duration}}$$

Column Chromatography and Radioimmunoassay

To quantify the concentration of CORT, DHT, and T present in plasma samples, column chromatography and radioimmunoassay were carried out according to the procedures outlined in Leary and Harris (2013). Briefly, plasma samples were incubated overnight with radiolabeled hormone (PerkinElmer, Inc. Hebron, Kentucky) for determination of recoveries for each sample. Steroids were then extracted from plasma using diethyl ether, dried under nitrogen gas at 40 °C, and resuspended in 10% ethyl acetate in iso-octane. Samples were then loaded onto diatomaceous earth columns containing a 3:1 diatomaceous earth:distilled water “glycol trap” and a 1:1 propanediol:ethylene glycol mixture. Mixtures of 10%, 20%, and 52% ethyl acetate in

iso-octane were then used to collect DHT, T and CORT, respectively. Fractions were dried under nitrogen and resuspended in phosphate buffered saline containing 0.3% gelatin for use in radioimmunoassay. Testosterone antibody was obtained from Fitzgerald Industries International, Inc (Acton, Massachusetts) and used for both T and DHT assays. CORT antibody was purchased from MP Biomedicals, LLC (Solon, Ohio). All samples were assayed in duplicate.

Statistical Analysis

Body condition was calculated by taking the residual values from the linear regression of the cubed root body mass divided by SIL (Howard and Young, 1998). This was performed to take into account the allometry associated with the relationship between body size and energy reserves. Body temperature was measured using a laser-point thermometer (Cen-Tech, Inc). Vocal effort, call duration, and intercall duration were adjusted to the mean ambient temperature of 24°C because variation in body temperature can influence these vocal attributes (Brown and Littlejohn, 1972; Gerhardt and Huber, 2002). This adjustment was accomplished by calculating residual values obtained from plotting vocal effort, call duration, and intercall duration against ambient temperature and using the equation of the regression line (Leary et al., 2008). Linear regression analysis was performed to examine the relationships among circulating hormone levels (DHT, T, and CORT), call parameters, and measures of body condition (SIL and weight) for calling host males and for satellite males that called after removal of the calling host and those that did not. One-way analysis of variance (ANOVA) was performed to compare the differences in means for the same variables measured in calling host males and satellite males.

RESULTS

Size, body condition, and circulating hormone levels in satellites and calling hosts

Satellite males were smaller than calling males ($F_{1,70} = 32.24$, $p = <0.0001$; Table 1) and in poorer body condition ($F_{1,70} = 6.64$, $p = 0.01$; Table 1). Additionally, satellites had significantly lower levels of circulating DHT ($F_{1,52} = 7.62$, $p = 0.008$; Table 1; Fig. 1) and higher levels of circulating CORT ($F_{1,52} = 6.06$, $p = 0.02$; Table 1; Fig. 1) relative to calling males; however, T levels did not differ between the two behavioral phenotypes ($F_{1,52} = 2.32$, $p = 0.13$; Table 1; Fig. 1). Still, total androgens (DHT+T) were significantly lower in satellite males compared to calling hosts ($F_{1,52} = 6.14$, $p = 0.02$; Table 1). The sample size for circulating hormone levels and the sample size for SIL and body condition differ because not enough plasma was obtained from some males for radioimmunoassay.

Circulating levels of T and DHT were not related to body condition ($r^2 = .0003$, $p = 0.92$; $r^2 = 0.003$, $p = 0.78$, respectively). There was a marginal negative correlation between CORT and body condition ($r^2 = 0.12$, $p = 0.06$; Fig. 2). However, DHT and T concentrations were positively correlated with size ($r^2 = 0.27$, $p = 0.005$; $r^2 = 0.22$, $p = 0.01$, respectively) indicating that large males have higher levels of circulating androgens.

Call parameters in satellites and calling hosts

The advertisement calls of satellite males that vocalized subsequent to host removal possessed higher average carrier frequencies in the low frequency peak of the call than calling host males ($F_{1,58} = 17.10$, $p = 0.0001$; Table 1). Satellite males that called after removal of the calling host also produced calls that were shorter in duration ($F_{1,61} = 15.99$, $p = 0.0002$; Table 1) and had a longer intercall duration ($F_{1,61} = 15.62$, $p = 0.0002$; Table 1). Hence, satellite males

that vocalized after host removal had lower calculated vocal effort than calling host males ($F_{1,61} = 41.62$, $p = <0.0001$; Table 1).

Relationships among call parameters, circulating hormone levels, and body condition

The low frequency peak of the advertisement call was negatively correlated with body condition ($r^2 = 0.14$, $p = 0.002$; Fig. 3) and positively correlated with CORT ($r^2 = 0.24$, $p = 0.0008$; Fig. 4). These correlations indicate that males producing advertisement calls with higher frequencies tend to be in poorer body condition and have higher circulating CORT levels. Call duration was negatively correlated with CORT ($r^2 = 0.09$, $p = 0.04$; Fig. 4), meaning that males with higher CORT levels have shorter calls. Vocal effort was not correlated with T ($r^2 = 0.05$, $p = 0.12$; Fig. 6), but positively correlated with body condition ($r^2 = 0.07$, $p = 0.03$; Fig. 7). These correlations show that vocal effort is not related to circulating T levels but males in better body condition invest more energy in calling. DHT was positively correlated with vocal effort ($r^2 = 0.11$, $p = 0.02$; Fig. 8), while CORT was negatively correlated with this variable ($r^2 = 0.15$, $p = 0.007$; Fig. 9). This indicates that males with increased vocal effort have higher circulating DHT and lower CORT levels.

Hormone profiles of calling and non-calling satellite males

I analyzed the circulating levels of DHT, T, and CORT in the satellite males of 27 satellite-caller associations. Nineteen out of 27 (70%) satellite males called and 8 out of 27 (30%) satellite males did not call subsequent to the removal of the calling host. ANOVA showed that the satellite males that called after removal of the calling host had significantly higher circulating levels of DHT than those that did not call ($F_{1,25} = 6.11$, $p = 0.02$; Fig. 10) but not higher levels of T ($F_{1,25} = 2.73$, $p = 0.11$; Fig. 10). Furthermore, total androgens (DHT+T) were

significantly higher in the satellite males that vocalized subsequent to host removal ($F_{1,25} = 5.69$, $p = 0.02$). The two groups of satellite males did not differ significantly in circulating levels of CORT ($F_{1,25} = 0.77$, $p = 0.38$; Fig. 10).

	N	Host Mean	Satellite Mean	F-value	P-value
Low Frequency (kHz)	60	0.80 ± .07	.94 ± .18	17.10	0.0001
Call Duration (ms)	63	156.44 ± 21.86	133.50 ± 23.19	15.99	0.0002
Intercall Duration (ms)	63	419.24 ± 70.89	507.28 ± 106.02	15.62	0.0002
Vocal Effort (%)	63	0.27 ± 0.04	0.21 ± 0.04	41.62	<0.0001
SVL (mm)	72	53.73 ± 4.42	47.62 ± 4.69	32.24	<0.0001
Weight (g)	72	9.32 ± 1.95	6.23 ± 2.09	41.82	<0.0001
Body Condition	72	0.001 ± 0.002	-0.001 ± 0.002	6.64	0.01
DHT (ng/mL)	54	34.67 ± 16.67	23.71 ± 12.11	7.62	0.008
T (ng/mL)	54	14.99 ± 8.22	11.51 ± 8.52	2.32	0.13
CORT (ng/mL)	54	2.23 ± 2.09	5.65 ± 6.89	6.06	0.02
Total Androgens (ng/mL)	54	49.66 ± 23.78	35.23 ± 19.86	6.14	0.02

Table 1: Sample sizes, means, standard errors, F, and p values for comparison between *Hyla cinerea* calling host and satellite males in terms of call parameters, body condition, and circulating hormone levels.

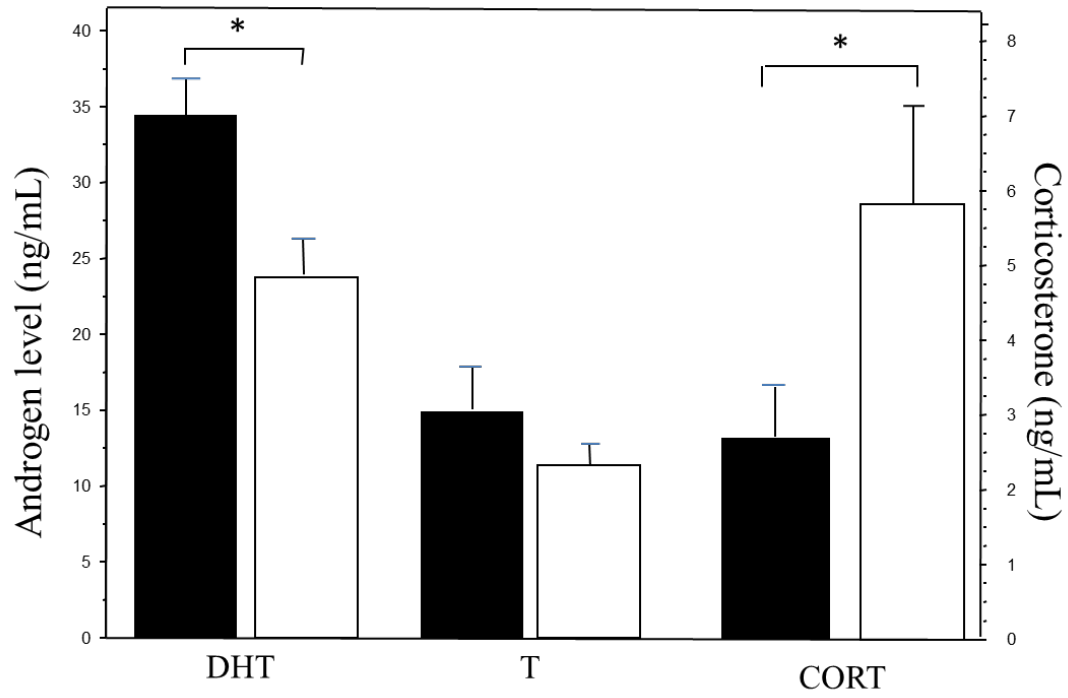


Figure 1: Bar graph highlighting the hormone differences between satellite males (white) and calling host males (black). Whiskers depict standard errors and asterisks denote significant differences.

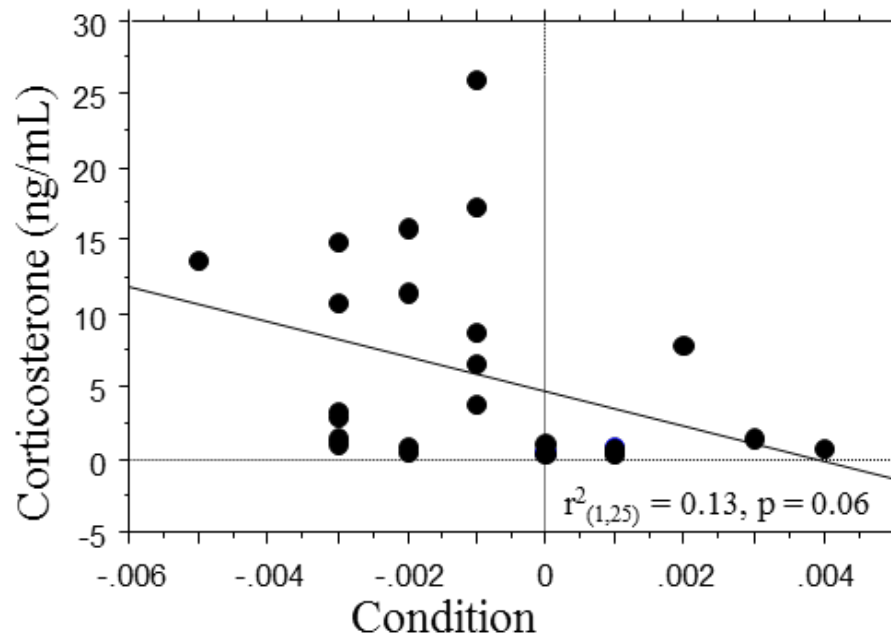


Figure 2: Linear regression showing the relationship between CORT and body condition.

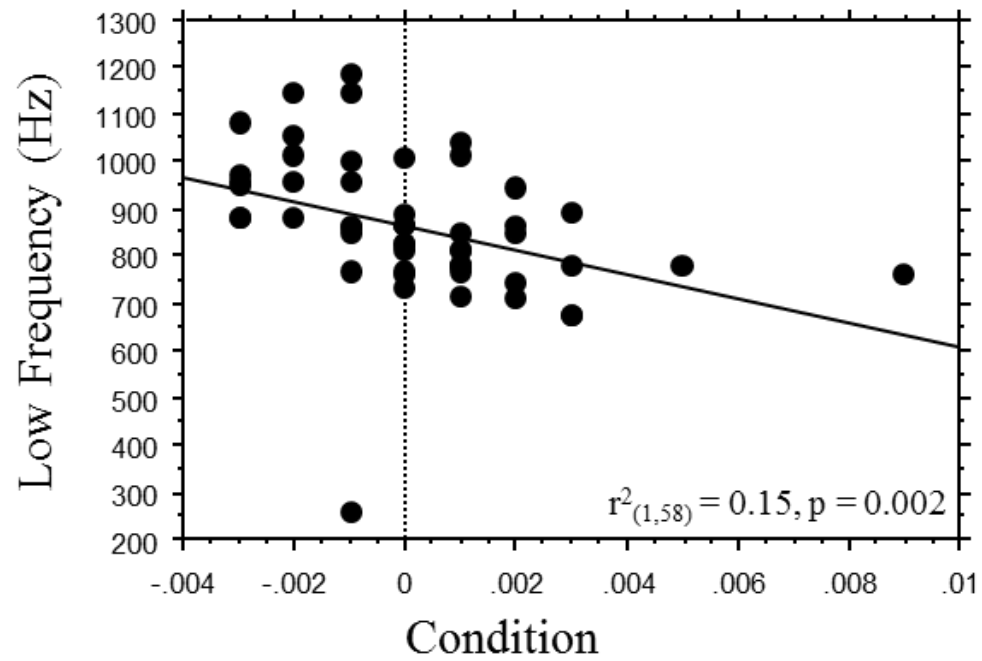


Figure 3: Linear regression showing the relationship between low frequency and body condition.

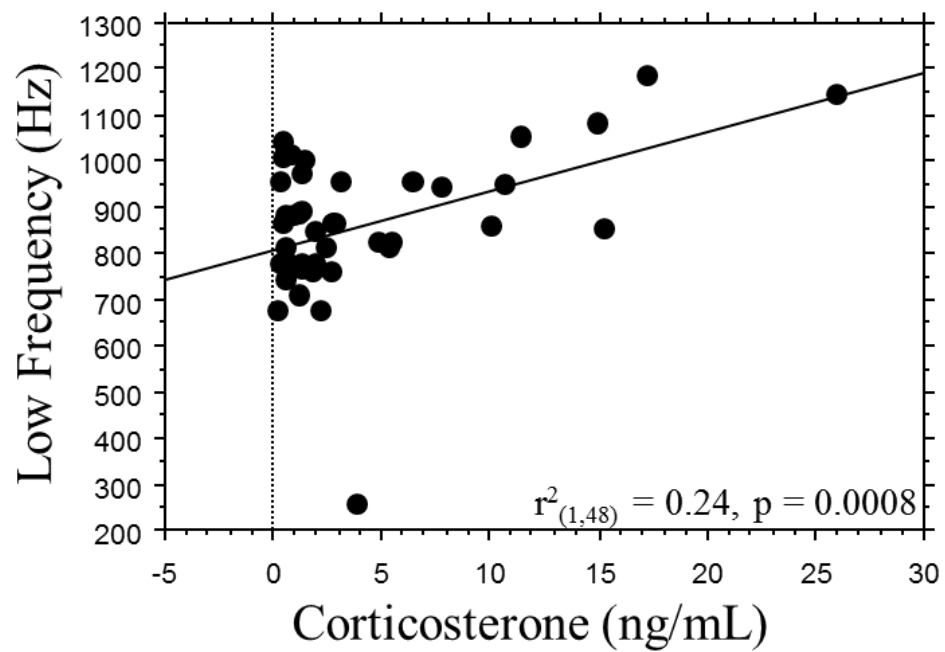


Figure 4: Linear regression showing the relationship between low frequency and circulating
CORT level.

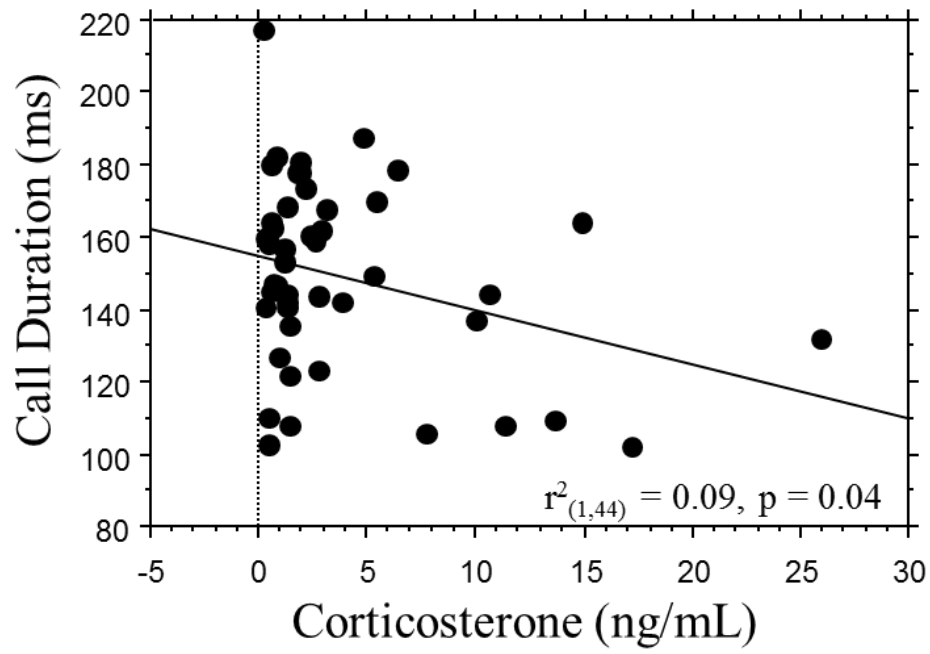


Figure 5: Linear regression showing the relationship between circulating CORT level and call duration.

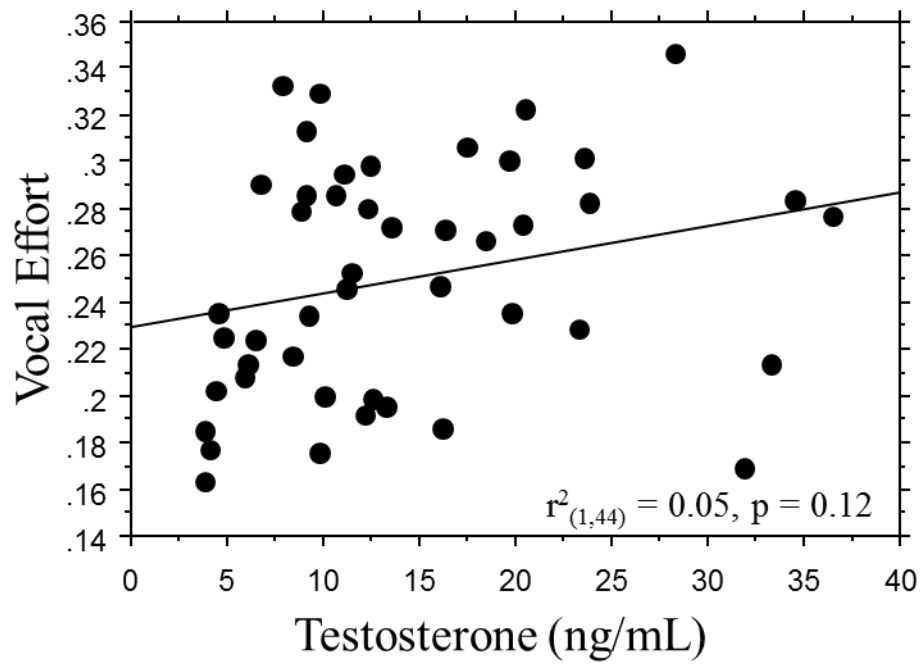


Figure 6: Linear regression showing the relationship between circulating T level and vocal effort.

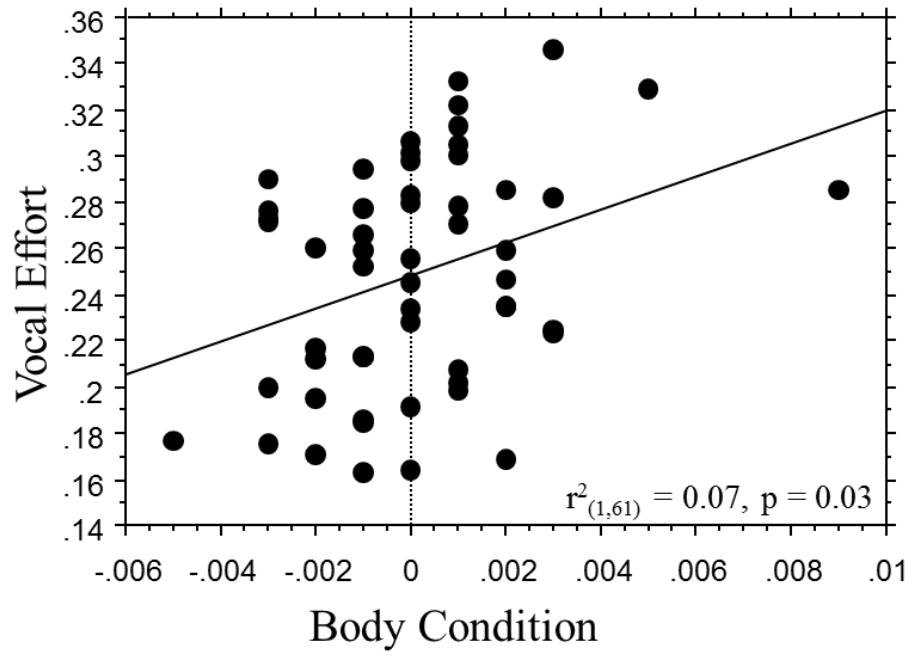


Figure 7: Linear regression showing the relationship between body condition and vocal effort.

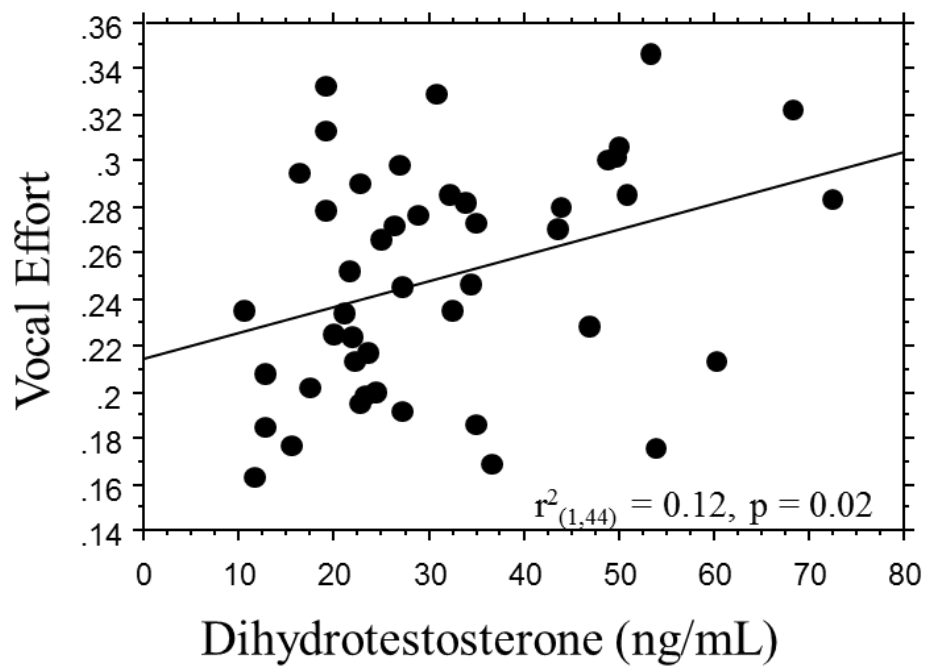


Figure 8: Linear regression showing the relationship between circulating DHT level and vocal effort.

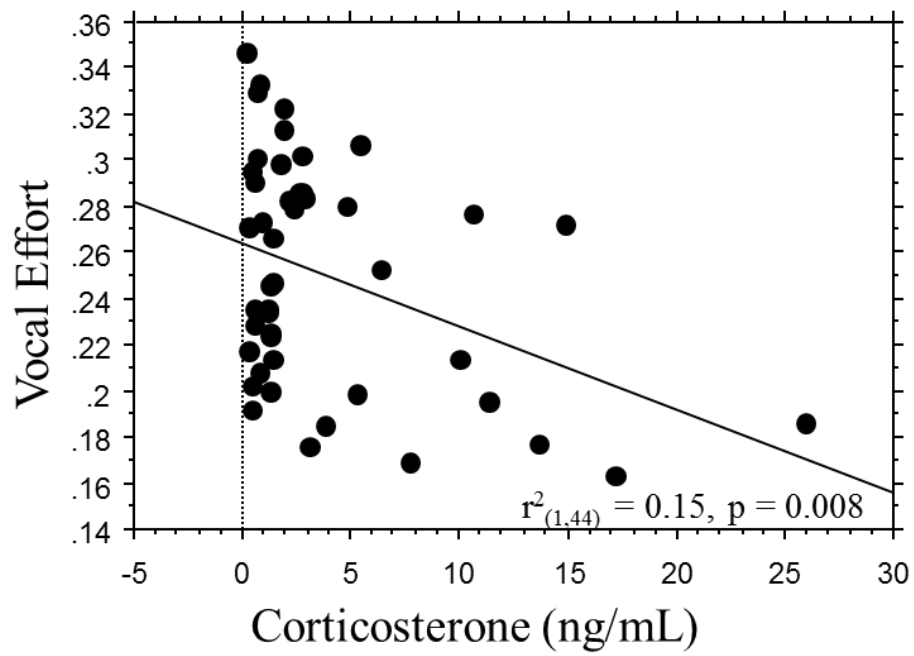


Figure 9: Linear regression showing the relationship between circulating CORT level and vocal effort.

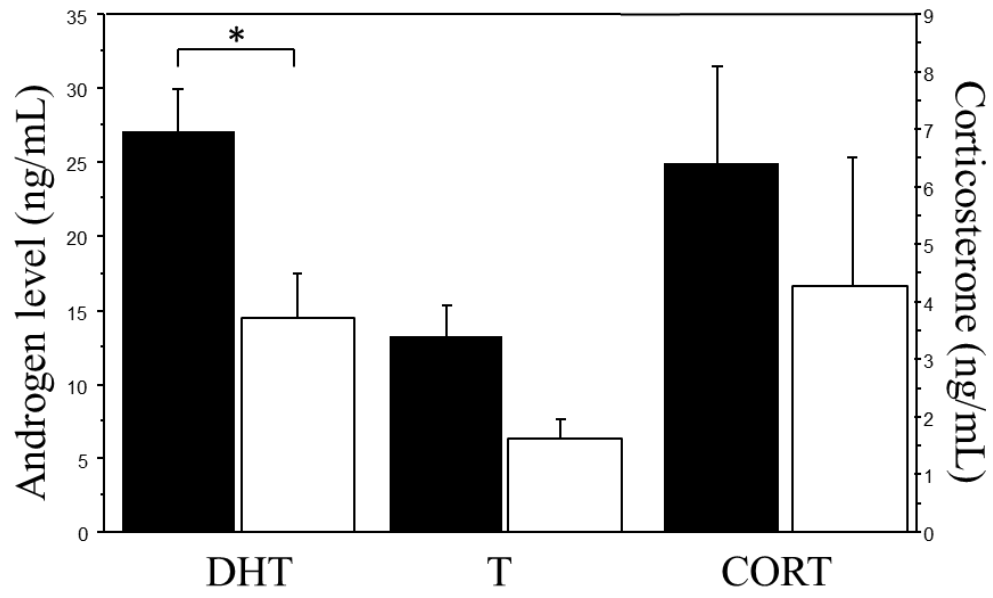


Figure 10: Bar graph highlighting the endocrine differences between satellite males that called subsequent to removal of the calling host (black) and those satellites that did not start calling after host removal (white). Whiskers depict standard errors and asterisks denote significant differences.

DISCUSSION

Previous research examining endocrine differences between satellite male and calling male *H. cinerea* showed that satellite males have significantly lower circulating levels of DHT and T and significantly higher levels of CORT (Leary and Harris, 2013). My results similarly show that satellite males have significantly lower levels of DHT and significantly higher levels of CORT; however, circulating levels of T were not higher in host males. The lack of significant difference in circulating T levels may be explained by the fact that I only sampled calling males that were associated with satellite males. The previously cited study involved the sampling of calling males found in association with satellites along with calling males not found in a satellite-caller association (Leary and Harris, 2013). Sampling a wider variety of calling males in this way may have resulted in discrepancies in circulating T levels.

Satellite males that vocalized subsequent to host removal were shown to have higher average carrier frequencies in the low frequency peak of the call than calling host males, suggesting that satellite males produce calls that are not as attractive to females as the advertisement calls of calling host males since females prefer calls of lower frequency (Humfeld, 2008). The low frequency peak was also shown to be negatively correlated with body condition and positively correlated with CORT. These correlations further support the idea that satellite males are expected to produce calls that are less attractive to females since satellite males have higher CORT levels and are in poorer body condition. Satellite males produced calls that were shorter in duration and had a longer intercall duration, which resulted in a lower calculated vocal effort. My results thus show that the satellite males that do call subsequent to host removal invest less energy in vocalization than calling host males.

Prior host removal experiments in anurans have not addressed the endocrine differences between satellite males that switch their mating tactic (e.g. begin to call) and those satellite males that do not start to call (Perrill et al., 1982; Humfeld, 2008). My results indicate that satellite males that called subsequent to host removal had significantly higher levels of DHT but levels of T and CORT did not differ from satellite males that did not begin to call. The Energetics-Hormone Vocalization (EHV) model (Emerson, 2001) predicts that higher androgen levels should increase vocal effort. Consistent with this prediction, I found that circulating levels of DHT and vocal effort were positively correlated. Circulating levels of CORT were not significantly different between the two groups of satellite males. This result was unexpected based on the EHV model, which predicts that circulating levels of CORT suppress calling behavior via negative effects on androgen production (Emerson, 2001). CORT level was, however, negatively correlated with vocal effort and call duration.

A problem that isn't fully understood is whether the transition between the satellite tactic and calling behavior in *H. cinerea* and other frogs is linked to changes in circulating hormone levels or whether small males represent a group with high CORT levels and low androgens that are predisposed to non-vocal behavior (Leary and Harris, 2013). One suggestion for future research into this problem would be to experimentally alter the circulating hormone levels of satellite males subsequent to host removal and see if calling behavior can be promoted. Furthermore, research into the mechanisms of action of these hormones at the cellular level could provide valuable insight into how these molecules function beyond knowing that they are important in the mediation of behavior. For example, it is known that CORT rapidly suppresses vocalizations in *Bufo cognatus* and *B. woodhousii* independently of changes in circulating

androgen levels, and this suggests that CORT has direct effects on the specific brain centers that control vocalization (Leary et al., 2006).

In summary, my results support the differences found in previous studies between satellite males and calling host males in terms of their endocrine profiles, call parameters, and measures of body condition. I expanded upon this research using host removal experiments and found that androgens may play an important role in the transition between satellite and calling behavior that is often observed upon removal of the calling host from a satellite-caller association. In conclusion, my results suggest that circulating hormone levels, especially DHT, and the relative attractiveness of other males may play a significant role in mediating mating tactic decisions in *H. cinerea*.

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